

Original Article

Systematic Screening of the Serotonin Receptor 1A (5-HT1A) Gene in Chronic Tinnitus

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Abstract Objective Chronic tinnitus is a highly prevalent condition and has been hypothesized to result from an innate disturbance in central nervous serotonergic transmission. Given the frequent comorbidity with major depression and anxiety, we argue that candidate genes for these disorders are likely to overlap. The present study addresses the gene encoding for the 5-HT1A receptor as a putative risk factor for tinnitus. **Methods** In 88 subjects with a diagnosis of chronic subjective tinnitus who underwent a detailed neurootological examination, the entire 5-HT1A gene was amplified using overlapping PCR products. Amplicons were custom sequenced bidirectionally and were screened for variants in multiple alignments against the human genome reference. **Results** We identified a synonymous C>T exchange at residue 184 (Pro) in 7/88 subjects, but detected no missense variants in the population under study. Specifically, the following residues were fully conserved: 16 (Pro), 22(Gly), 28(Ile), 98 (Val), 220(Arg), 267 (Val), 273 (Gly), and 418 (Asn). **Discussion** The present data count against the causation of chronic tinnitus by a change in the 5-HT1A receptor's amino acid sequence. However, the allele frequency for the 184Pro minor allele (0.04) reached twice the frequency reported in control cohorts from the same ethnicity. Additional investigations are invited to clarify the role of the 5-HT1A polymorphism in larger samples, and to control for comorbid affective disorders.

Keywords 5-HT1A; Gene; Tinnitus

Introduction

Chronic tinnitus is a highly prevalent and often incapacitating condition that has been estimated to affect some 130 million Chinese and 40 million Americans^[1, 2]. While information from family studies is still lacking and reliable estimates of the heritability associated with tinnitus are not currently available, anecdotal evidence suggests that as many as one-third of patients may suffer from a familial susceptibility to the disorder (Kleinjung et al., unpublished data). Given the frequent co-occurrence of tinnitus and affective disorders, it has been proposed that some overlap exists regarding specific vulnerability factors in a subset of patients. This notion is supported by clinical

benefits experienced by subjects complaining of chronic tinnitus after the intake of anxiolytic and antidepressant medication^[3].

Specifically, several lines of evidence suggest that the serotonin receptor 1A (5-HT1A) plays a key role in modulating susceptibility to affective disorders^[4], and is equally relevant to the processing of auditory stimuli^[5]. 5-HT1A receptors are coupled to G-proteins and feature seven transmembrane domains. They are widely expressed throughout the brain, including the auditory nuclei^[6]. In animals, 5-HT1A receptor agonists control neuronal firing rates in the medial vestibular nucleus^[7]. Genetic variation in the 5-HT1A gene on human chromosome 5q11 is associated with the P2 component of auditory evoked potentials in patients with major depression^[8]. In the light of these findings, we hypothesized that 5-HT1A gene variation may account for an increased risk of developing chronic tinnitus and conducted a systematic screening of the gene in affected individuals.

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Methods

In 88 subjects (63 men and 25 women, age 50.5 ± 13.6 yrs, mean \pm SD) a diagnosis of chronic subjective tinnitus (mean duration 6.6 yrs, range 1-35 yrs) was confirmed by a detailed neurootological examination including otoscopy, stapedius reflexes, middle ear pressure measurements, pure tone audiometry, tinnitus pitch and loudness matches. Those patients with a history of vestibular schwannoma, Meniere's disease, or pathological middle ear conditions were excluded. Participants in the present study were recruited consecutively at a tinnitus clinic. Tinnitus severity was assessed by the Tinnitus Questionnaire^[9] and averaged 43.4 ± 2.3 (mean \pm SEM) out of 84 points (N = 84). By this measure, tinnitus was considered mild in 29%, moderate in 26%, severe in 16%, and extreme in 29% of subjects investigated. Genomic DNA was extracted from lymphocytes using standard procedures prior to amplification of the entire 5-HT1A gene. Two overlapping amplicons were generated (726 and 750bp, respectively) by the following oligomers: 5'-ATTCCCTTCCTCCGAACTT-3' (forward), 5'-AAAGCTCCAAAGGTGGAATAGATAG-3'(reverse), 5' -TGCACCATTAGCAAGGATCA-3' (forward), 5'-GGATCCTGTAGCCTCGACTG-3'(reverse). PCR products were custom sequenced bidirectionally and were screened for variants in multiple alignments against the human genome reference(Build 35, chr5 clone RP11-158J3) using Lasergene V4.0(DNASTar Inc.). Prism V2.01 (GraphPad Software Inc.) was used for descriptive statistics and for comparison of mean scores on the Tinnitus Questionnaire (TQ) by unpaired t-tests.

Results

We identified a synonymous C>T exchange (SNP-ID #rs1800043, Fig. 1) at residue 184 (Pro) in 7 out of 88 subjects at a frequency of 0.04 for the lesser T-allele. The genotype distribution did not deviate from the Hardy-Weinberg equilibrium($p > 0.69$, Pearson's χ^2 -test). No known or novel missense variants were encountered in the population under study(Table 1) and the following reportedly polymorphic residues were fully conserved: 16 (Pro), 22 (Gly), 28 (Ile), 98 (Val), 220 (Arg), 267(Val), 273 (Gly), and 418 (Asn). Subjects carrying the T-allele rated their tinnitus as more severe (mean TQ score = 52.7 ± 8.6) than did subjects carrying only the wild type allele (mean TQ score = 42.7 ± 2.3), but this difference did not reach significance ($p = 0.26$, $t = 1.14$, $df = 82$).

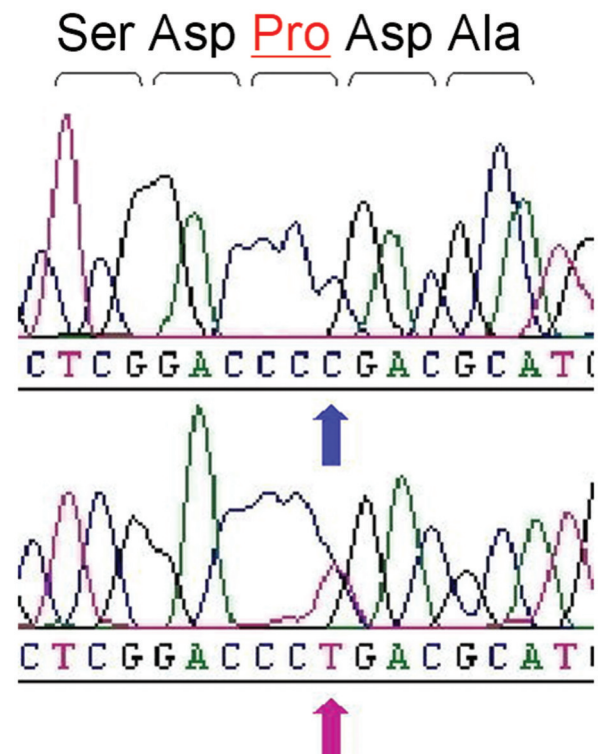


Fig. 1 Chromatograms of the homozygous wildtype (top) and heterozygous variant(bottom) sequence at 5-HT1A residue 184Pro.

Table 1. Available data on 5-HT1A gene variants including coding variants (shaded rows) which were systematically screened for in the present study. Of these, only one variant was identified (Pro184Pro) in subjects with chronic tinnitus.

published 5-HT1A variants and dbSNP identifiers	population frequency reported in the literature
C(-1019)G = rs6295	~ 0.50 (Cargill et al., 1999)
C(-581)A = rs1800048	< 0.01 (Kawanishi et al., 1998)
A(-480) = rs1799733	< 0.01 (Kawanishi et al., 1998)
G(-321)C = rs1800047	< 0.01 (Kawanishi et al., 1998)
C(-152)G = rs1800046	< 0.01 (Kawanishi et al., 1998)
T(-51)C = rs1800045	< 0.01 (Kawanishi et al., 1998)
Pro16Leu = rs1800041	~ 0.04 (Kawanishi et al., 1998)
Gly22Ser = rs1799920	< 0.01 (Nakhai et al., 1995)
Ile28Val = rs1799921	< 0.01 (Marshall et al., 1999)
Val98Val = rs6294	~ 0.11 (Glatt et al., 2004)
Pro184Pro = rs1800043	~ 0.02 (Glatt et al., 2004)
Arg220Leu = rs1800044	< 0.01 (Ng et al., 2002)
Val267Met	< 0.01 (Glatt et al., 2004)
Gly273Asp = rs1800042	~ 0.06 (Sunyaev et al., 2001)
Asn418Lys	< 0.01 (Lam et al., 1996)

Discussion

The present data count against the causation of chronic tinnitus by a change in the 5-HT1A receptor's amino acid sequence. It cannot be excluded, however, that the above coding polymorphism is associated with the disease and is not functionally neutral. Up to one quarter of synonymous coding variants may lead to profound changes in splicing patterns^[10], and C/T transversions are more frequently associated with human disease than are G/A transversions. This strand asymmetry has been shown to reflect selective pressure in four-fold degenerate codons, to which belongs proline^[11]. Alternatively, the variant may be in linkage disequilibrium with regulatory variants further upstream of the transcriptional start site. So far, all previous reports that have addressed prevalence of the variant T-allele at residue 184 in healthy Caucasians^[12, 13], have provided lower frequencies ($f_{(T)} = 0.01$ and $f_{(T)} = 0.02$, respectively). In healthy Asian individuals, the allele frequency reported was marginally higher ($f_{(T)} = 0.03$,^[14]) but did not reach the frequency ascertained in the present investigation of tinnitus sufferers. Future studies will therefore need to address in further detail genotype and allele distributions in relation to comorbid affective disorders, and to specific tinnitus etiologies.

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